Fluoride uptake by cavity walls from a fluoride-containing amalgam *in vitro*

An electron microprobe analysis

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The aim of this study was to register the fluoride distribution in cavity walls following exposure to a fluoride-containing amalgam *in vitro*.

Class V cavities were prepared on buccal and lingual surfaces in ten extracted premolars and filled with a fluoride-containing (experimental) or a conventional (control) amalgam. After storing the teeth in artificial saliva for three months, the fillings were removed and the cavity walls analyzed with an electron microprobe.

The cavity walls which had been exposed to the conventional amalgam did not show F concentrations above the detection limit (0.15 %). In the experimental group, however, more than 60 % of the analyses of the enamel walls revealed fluoride concentrations greater than 0.15 %, the values ranging from 0.2 to 1.7 %. In the dentin walls fluoride concentrations of 0.2 – 1.9 % were measured. However, 80 % of the analyses revealed values between 0.9 and 1.5 %. The depth of the zone of increased F concentration in enamel and dentin walls was on the average 27 and 108 µm, respectively.

Although the concentrations should only be regarded as semiquantitative values, they indicate that considerable amounts of fluoride are deposited in the cavity walls from the fluoride-containing amalgam.

Key-words: Filling materials; fluoride analysis; dentin; enamel

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Small amounts of fluoride added to amalgam seem to have no significant deleterious effect on the physical and chemical properties of the filling material (2, 15, 17, 21). On the other hand, interesting biological effects have been reported, i.e. reduced solubility of the enamel adjacent to fluoride-containing amalgam restorations (15), a lower incidence of secondary caries (21) and *in vitro* inhibiting effect on the development of cavity wall lesions around such fillings (29). Such effects may be explained by the deposition of fluoride in the dental hard tissue around the restorations, or the presence of fluoride ions in the microspace or close to the restorations under conditions favorable to the development of caries. Consequently, the amount of fluoride deposited in enamel and dentin around fluoride-amalgam fillings, may give some indication of the caries-inhibiting qualities of this material.

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Fluoride uptake by enamel and dentin from a fluoride-containing amalgam has been demonstrated in some studies (10, 16, 30). In two of these studies (10, 30) only the outermost 9 μm of enamel and 12 μm of dentin surfaces which had been exposed to the amalgam could be anlyzed by the method used. The results, however, indicated considerable amounts of fluoride deposited in these zones.

With the electron microprobe the distribution of fluoride throughout the whole tissue, except for the outermost 4–5 μm, can be assessed. Fluoride uptake by cavity walls from the same fluoride-amalgam was measured by this method in one study (16), but incomplete information was given regarding the amount of fluoride deposited and the depth of the zones showing fluoride uptake.

Since this amalgam was found to inhibit development of cavity wall lesions during a three-month experiment *in vitro* (29), the present study aimed to register the amount of fluoride deposited in the cavity walls under similar experimental conditions.

MATERIAL AND METHODS

Ten premolars, extracted for orthodontic reasons, were used in this study. The teeth were clinically sound and had been stored in 10 % buffered formalin solution since extraction. Each tooth was divided in two parts by longitudinal mesio-distal sectioning. Class V cavities were prepared on each buccal and lingual surface using a water cooled air rotor, and finished with diamonds (80,000 rpm) and finishing burs (15,000 rpm), rinsed with water and dried with compressed air. One cavity from each tooth was filled with a fluoride-containing amalgam (Fluor Alloy®) (experimental group) and the other with a conventional amalgam (New True Dentalloy®) (control group). The manufacturers' instructions were followed and the materials were condensed using hand instruments.

Each tooth half was then stored at 37°C in 20 ml of artificial saliva changed weekly for 3 months. The artificial saliva, prepared according to a formula proposed by Fusayama et al. (11) and later modified by Meyer et al. (19), had a pH of 5.2. Following the experimental period, 3 or 4 150–200 μm-thick longitudinal sections, passing in bucco-lingual direction through the cavity, were prepared from each tooth half. The filling material was then removed, and the sections were placed in an ultrasonic cleaner for two minutes to remove remnants of amalgam attached to the dental tissue. The sections were polished with AB Alpha Polishing Alumina® No. 2, glued to Specifix® blocks and covered with a thin layer of carbon before being analyzed with an electron microprobe.

Electron Microprobe Analysis

The fluoride analyses were carried out in an Applied Research Laboratories electron microprobe SEMQ which was operated at 10 kV with a sample current of 50 nA on brass. The diameter of the area of X-ray generation, which is always larger than the diameter of the electron beam on the specimen surface, was approximately 2 μm.

Line scans of the electron beam were made at right angles to the specimen surface, and the Kα emission of fluoride, calcium and phosphorus was recorded simultaneously. Ca and P analyses were performed in order to determine the exact position of the edge of the cavity walls. The scan speeds

ranged from 20 μm/min to 146 μm/min, and the time constant was kept at 0.5 sec. The recorder was calibrated by point analyses when the X-ray intensity was recorded on a scaler. Spectral interferences of higher order were eliminated by the use of pulse height analysis.

One might suspect that some of the FKα radiation originated from particles of filling material. In order to identify such particles, some analyses of tin were performed.

A fluorapatite mineral containing 38.94 % (by weight) Ca, 17.77 % P and 3.85 % F was used as a standard.

The measured intensities were corrected for background and dead time loss, and the concentrations of the elements were estimated assuming a linear relation between concentrations and intensities in the specimens as compared to those in the standard. To test the validity of this assumption some of the data were further corrected for absorption, fluorescence and atomic number effects by means of a modified version of a correction program originally written by Dullum (6). The correction program was written and tested for analysis of the relatively light elements in felspar, pyroxene and olivine minerals where a large amount of oxygen is present. The program has, however, been used successfully for a wide variety of specimens. In order to work satisfactorily it requires, however, that the X rays are produced in a homogeneous volume. This is probably not always the case in this study.

The following procedures were used:

For absorption: Bishop's model (3) with Heinrich's model for the Lenard coefficient (14) and with a value of the parameter h evaluated by Love (18).

For the atomic number effect: Philibert & Tixier's atomic number correction (22) with Springer's (25) expression for the effective current factor and Zeller's (31) expression for the mean ionization potential, as described by Ruste & Gantois (24).

For characteristic fluorescence: Büchner & Stienen's (5) procedure, modified with the Bishop absorption model. Where this procedure is not valid, Reed's (23) procedure was used.

For continuous fluorescence: Springer's (26) model.

Other elements than F, Ca and P were not analyzed, but in order to take into account their influence upon the measurements, the contents of Mg, Na, Cl and C were estimated according to data from chemical analysis (20) and the content of O was programmed to be introduced by difference to give a total of 100 %.

The calculated correction factors for F, Ca and P that could be calculated on the basis of physical and instrumental data were so close to 1.0 that there was little need to take them into account. Other sources of error seem to be more important, as will be discussed later.

RESULTS

The pH of the artificial saliva varied between 5.2 and 5.8 during the course of the experiment.

Control groups

Expectedly, fluoride analysis of the cavities which had been filled with a conventional amalgam did not show F concentrations above the detection limit (0.15 %) (Figs. 1, 2). For this reason, only four analyses were performed in each of these enamel and dentin cavity walls.

Experimental groups

Some analyses revealed very high fluoride and tin concentrations near

the surface of the experimental cavity walls, indicating the presence of particles of the filling material in these areas. Such remnants of fluoride-amalgam caused fluorescence (1), which sometimes invalidated the fluoride analysis of the cavity walls. For this reason some analyses had to be excluded.

Nine to eleven usable analyses were available from each enamel and dentin cavity wall in nine specimens. In one specimen, seventeen analyses from the enamel wall and twelve from the dentin wall were included. More than half of the scans of enamel walls showed F concentrations greater than 0,15 %, while all analyses of the dentin walls showed concentrations greater than this value. The concentrations varied considerably within each specimen.

More than 40 % of the scans from the enamel walls revealed fluoride concentrations of 0.2–0.4 %, while 23 % showed concentrations in the range 0.4–1.7 %. The remaining analyses indicated F concentrations lower than the detection limit (Fig. 1). In the dentin walls, F concentrations up to 1.9 % were registered. Most of the analyses (80 %), however, showed concentrations from 0.9 to 1.5 % (Fig. 2).

Representative electron microprobe scans from enamel and dentin cavity walls are shown in Figs. 3 and 4. The concentration profiles of F reached maximum values at the border of the cavity walls sloping downwards to the normal level at varying depths. The width of the layer showing increased F concentration in the enamel walls was 27 ± 27 μm (mean value ± S.D.) and in the dentin walls 108 ± 54 μm.

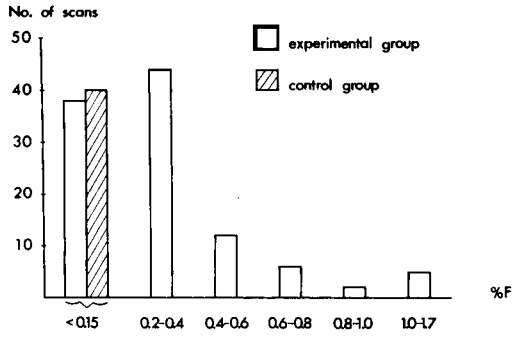

Fig. 1. Fluoride concentrations in enamel cavity walls exposed to a fluoride-containing amalgam (experimental group) and a conventional amalgam (control group) for three months *in vitro*. The bars indicate number of scans showing fluoride values within each concentration range.

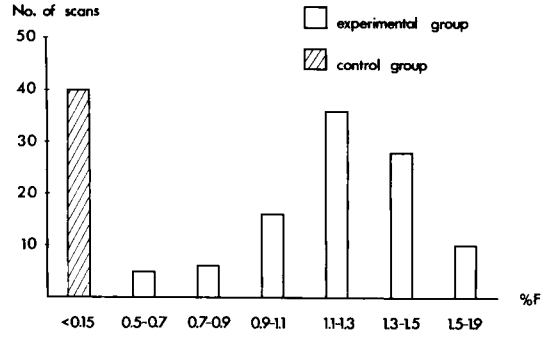

Fig. 2. Fluoride concentrations in dentin cavity walls exposed to a fluoride-containing amalgam (experimental group) and a conventional amalgam (control group) for three months *in vitro*. The bars indicate number of scans showing fluoride values within each concentration range.

DISCUSSION

It is most likely that the fluoride-containing amalgam has optimal effect as a local source of fluoride under conditions favorable to the development of carious lesions. Fluoride uptake under low pH conditions was therefore considered interesting, especially since the amalgam had demonstrated considerable inhibiting effect on the development of cavity wall lesions *in vitro* (29). Using the artificial saliva, an acidic experimental condition was created. In addition, this medium affects the corrosion process of dental alloys in a manner similar to that of natural saliva (19). Corrosion may be of importance when

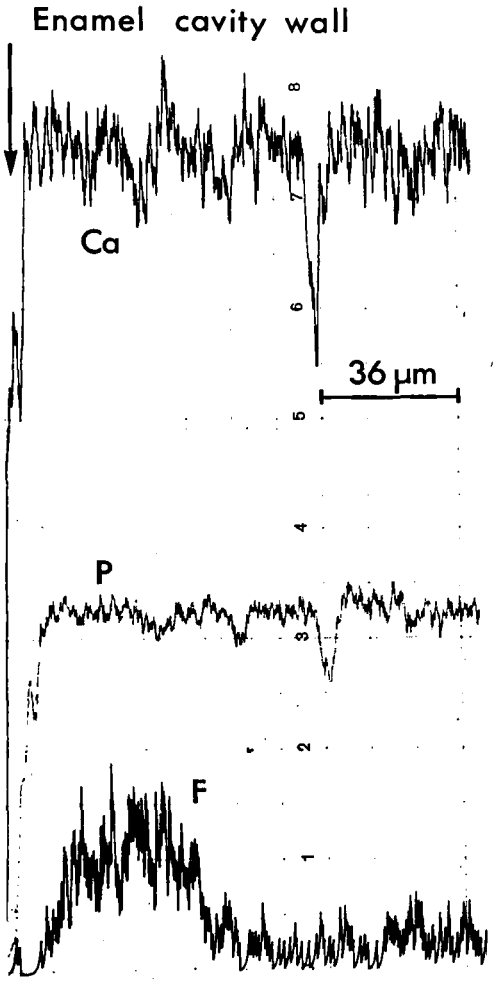

Fig. 3. Linear scans showing concentration profiles of calcium, phosphorus and fluoride in an enamel cavity wall exposed to a fluoride-containing amalgam for three months *in vitro*. Note elevation of fluoride profiles in the outer zone of the tissue.

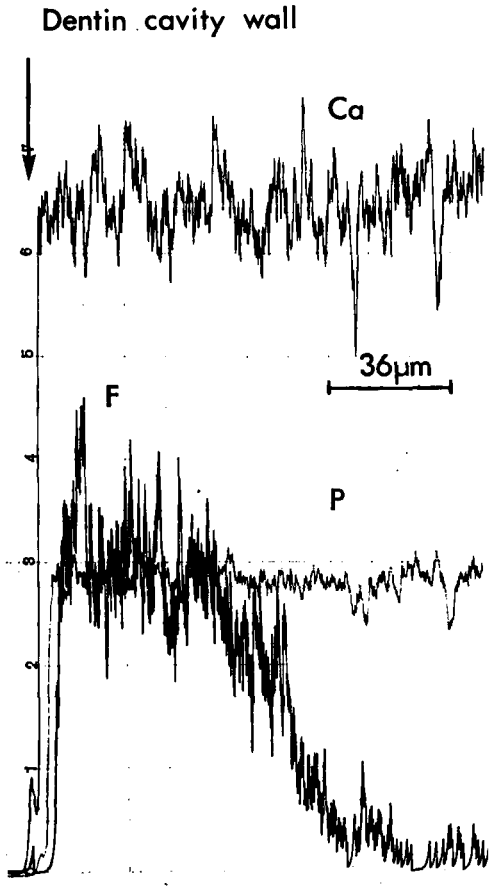

Fig. 4. Linear scans showing concentration profiles of calcium, phosphorus and fluoride in a dentin cavity wall exposed to a fluoride-containing amalgam for three months *in vitro*. Note elevation of fluoride profiles in the outer zone of the tissue.

the fluoride release from the material is concerned.

For several reasons the concentrations reported in this study should be regarded as semiquantitative values only. In the analysis of enamel quite reliable results can be expected (13) because of the homogeneity of the specimen and the compositional similarity between the specimen and the fluorapatite standard.

In the case of dentin the situation is less favorable. Although the calculated correction factors are close to 1.0, the concentrations of Ca and P are some 15 % higher than those generally found by chemical methods. This is in agreement with earlier findings (7). This discrepancy is probably due to several effects, such as sputtering of organic material by the electron beam (7, 8, 9), thermal conditions, as well as the low and inhomogeneous density of the dentin. The effective X-ray absorption will be reduced by a low density matrix,

thus increasing the apparent concentrations. As the absorption correction is even more critical for the F Kα radiation than the Ca Kα and P Kα, it is expected that the measured fluoride concentrations in dentin are affected to a greater extent.

Fluoride concentrations below 0.15 % were ignored, and no steps were taken to improve this rather crude estimate of a practical lower detection limit (1).

The considerable fluoride uptake by enamel and dentin from the fluoride-containing amalgam is in accordance with results from a previous study, a proton microprobe analysis (30). However, in that study only the outermost 12 μm of the tissue could be analyzed and, consequently, the distribution of fluoride throughout the whole tissue could not be assessed. Using the electron microprobe, remarkably wide zones showing fluoride increase were demonstrated. Conceivably, this deep penetration of fluoride could have resulted from the formation of hydrofluoric acid (HF) at the local site. Hydrofluoric acid is known to improve penetration of fluoride into enamel (4). However, very small amounts of HF dissociate in a NaF solution at pH 5.0 (4) and this may also have been the case under the experimental conditions of this study.

The great variations in fluoride concentration as well as in the depth of zones showing fluoride increase observed within each specimen, might be due to an unequal distribution of fluoride in the filling material rather than to preexisting chemical or structural variations within the hard tissues of the cavity wall. The greater uptake and deeper penetration of fluoride observed in dentin than in enamel is in accordance with previous observations, and can be explained by the differences in structure of these tissues (27, 30).

Compared to silicate cement, which also has caries-inhibiting properties, only small amounts of fluoride are released from the fluoride-containing amalgam to artificial saliva (28). Nevertheless, in the present study the fluoride uptake by enamel and dentin was nearly half of that observed from silicate cement restorations (27). In addition, experimental cavity wall lesions around fillings of these materials had similar characteristics (12, 29). Even though the results are not fully comparable due to small differences in experimental conditions, they indicate that these materials have similar properties in the sense that similar chemical and structural changes occur in the cavity wall.

Promising biological effects of fluoride-containing amalgam have been reported. However, most of the studies have been undertaken *in vitro*. Therefore, further clinical studies are necessary to evaluate the caries-inhibiting effect of this material.

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